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Protective Effect of Apigenin on Ovarian Follicles in Polycystic Ovary Syndrome-Induced Rats

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ABSTRACT

Background and objectives: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders that affect fertility. In this syndrome, the rate of fibrotic tissue formation and structures such as collagen increases. This study intended to evaluate protective effect of apigenin on ovarian follicles in PCOS-induced female Wistar rats.

Methods: In this experimental study, 60 adult Wistar rats (weight: 200-250 g) were used. A vaginal test was performed to confirm induction of PCOS. Then, the rats were randomly divided into four groups: 1) control, 2) PCOS, 3) PCOS+apigenin (10 mg/kg), and 4) PCOS+apigenin (20 mg/kg). The rats in the experimental groups received *apigenin solution via intrapertoneal injection for 14 days. Next,* the ovarian tissue of animals was removed and subjected to histological studies. Data were analyzed using one-way ANOVA and Tukey's post hoc test. Statistical analysis of data was carried out in SPSS (version 20), and significance level was set to 0.05.

Results: The number of secondary cystic follicles in groups treated with apigenin (10 and 20 mg/kg) decreased significantly compared to the PCOS group (group2) ($P \leq 0.05$). Despite the reduction in the number of follicles, this reduction for the primary follicles was not statistically significant. Moreover, treatment with apigenin had no significant effect on the number of graph follicles ($p \geq 0.05$).

Conclusion: Our findings indicate that apigenin might be useful in controlling PCOS. Therefore, it may be suggested as a supplement to improve fertility of patients with PCOS.

Keywords: Apigenin, Wistar Rats, Polycystic Ovary Syndrome, Ovarian Follicle.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders in women, affecting 6-10% of women in the reproductive age. This syndrome causes infertility and glucose intolerance in early and middle adulthood and causes diabetes mellitus and cardiovascular disease at older ages. The primary role of follicle is oocyte support. From birth, the ovaries of female humans contain a number of immature, primordial follicles. These follicles each contain a similarly immature primary oocyte. At puberty, clutches of follicles begin folliculogenesis, entering a growth pattern that ends in death (apoptosis) or in ovulation (the process through which the oocyte leaves the follicle). During follicular development, primordial follicles undergo a series of critical changes in character, both histologically and hormonally. First, they change into primary follicles and later into secondary follicles. During transition to tertiary or antral stage, the follicles become dependent on hormones, particularly follicle-stimulating hormone (FSH), which causes a substantial increase in their growth rate. The late tertiary or pre-ovulatory follicle ruptures and discharges the oocyte (that has become a secondary oocyte), ending folliculogenesis.

Development of PCOS is associated with hyperandrogenism and insulin resistance or insufficiency (1) and can also cause anovulation and infertility (2). In PCOS, gonadotropin-releasing hormone fluctuations increase in the blood, which leads luteinizing hormone (LH) elevation in the blood, followed by an increase in the concentration of steroids. Following an increase in LH and LH/FSH ratio in PCOS, estrogen levels increase, but progesterone levels decrease or remain unchanged. In addition, the levels of dehydroepiandrosterone and other androgens increase (2). The histological features of PCOS include ovarian enlargement, thickening of the theca layer, increase in the number of follicles and follicular sub-capsular cysts, hyperplasia, hardening of the ovarian stroma, premature luteinization of theca cells—hyperandrogenism, and increased fat mass. With long-term inflammation, the principal cells are destroyed, fibrocytes and fibroblasts are formed, and the production and density of collagen increase in the tissue (3). Fibrogenesis is one of the disorders that occur in the ovaries. The process of fibrogenesis is associated with high production of collagen and stromal tissues by fibrocytes and fibroblasts. Consequences of fibrogenesis include excessive deposition of extracellular matrix and decreased functional cells. Ovarian fibrogenesis is associated with various ovarian diseases, including ovarian cysts, PCOS, and premature ovarian failure. Patients with ovarian fibrosis respond less to infertility treatment (4). Several methods have been suggested to control PCOS. Medication is an essential step in improving the symptoms of PCOS. Treatment with chemical drugs such as metformin and tamoxifen had side effects but today many herbal medicines have potential effects on PCOS (5-7). Apigenin (4'.5.7 trihydroxy flavone) is a non-toxic and non-mutagenic flavonoid with molecular formula $C_{15}H_{10}O_5$ found in many fruits and vegetables (8). It has been argued that some flavonoids may have anticancer, anti-inflammatory, and antioxidant properties (9). Recent reports have shown that the antioxidant and antitumor properties of apigenin are due to its anti-angiogenic effects. Given the main role of angiogenesis and fibrogenesis in development of PCOS, apigenin can be potentially used for treatment of this syndrome (10, 11). Therefore, this study was performed to evaluate protective effects of apigenin on ovarian follicles in a rat model of PCOS.

MATERIALS AND METHODS

A total of 60 adult, female Wistar rats weighing 200-250 g were obtained from Pasteur Institute of Iran. The animals were kept under standard conditions (21 ± 2 ^{o}C, 12-hours light/dark cycle). The study was approved by the Ethics Committee and Deputy of Research and Development of Tehran University of Medical Sciences (ethical code: IR.TUMS.MEDICINE.REC.1398.915).

After a one-week adaptation period, all rats were randomly divided into four groups (n=15 per group). Group 1 was considered as normal control and did not receive any intervention/medication. PCOS was induced by intraperitoneal injection of estradiol valerate (4 mg/kg). A vaginal smear test was performed to confirm PCOS induction. For this purpose, 50 µl of 0.9% normal saline was injected into the vagina of each animal using a sampler; then, the equal amount was sucked

and placed on a slide. After drying, the samples were observed under a light microscope under 10× magnification. Next, different stages of the estrous cycle were identified based on classical cellular characteristics. This process was carried out every morning for three days. Animals that showed four regular and continuous estrous cycles were considered as normal in terms of a sexual cycle and entered the intervention phase. Thirty days after PCOS induction, rats in group 2 received daily apigenin solution (dissolved in 2% alcohol) intraperitoneally for 14 days. Rats in groups 3 and 4 received 10 mg/kg and 20 mg/kg apigenin solution for 14 days, respectively (12). In the next stage, ovaries of each rat were removed and fixed in modified Davidson fixative solution (30% formaldehyde, 15% ethanol, 5% glacial acetic acid, and 50% distilled water) for seven days. Then, the samples were washed with phosphate buffer saline, dehydrated, and embedded into wax blocks. The Masson's trichrome staining was performed to evaluate histological parameters such as the number of primary, secondary, graph, and cystic follicles. For all histological studies, 20 µm was cut from all sections.

From each group, 20 sections were examined, and the follicles were counted. Finally, the Micrometric SE Premium software was used to count the follicles and measure their dimensions.

Data were reported as means ± standard deviation. One-way ANOVA and Tukey's post hoc test were used to compare parameters between the study groups. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software (version 20).

RESULTS

Effect of different doses of apigenin on the number of primary follicles

The number of primary follicles in the PCOS group (group 2) was significantly lower compared to the control group ($p \leq 0.05$). Administration of apigenin at different doses had no significant effect on the number of follicles ($p \geq 0.05$) (Figure 1).

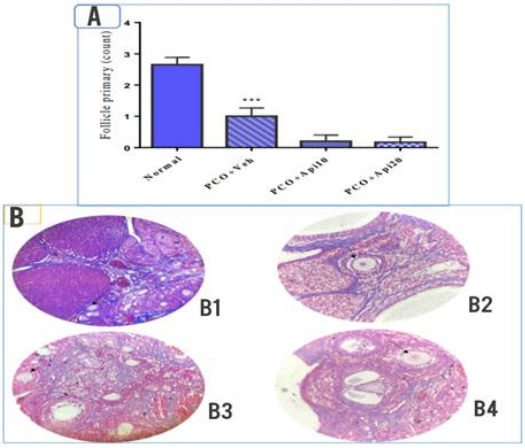

Figure 1- The effect of different doses of apigenin on primary follicles in ovarian tissue of polycystic rats. A) The number of primary follicles in the experimental groups. B) Tissue section of a polycystic ovary of a rat after Masson's trichrome staining (10× magnification). B1: control group, B2: PCOS group, B3: PCOS+ apigenin (10 mg/kg), B4: PCOS+ apigenin (20 mg/kg). * Significant difference compared to the control group.**

Effect of different doses of apigenin on the number of secondary follicles

The number of secondary follicles in the PCOS group (group 2) was significantly higher than in the control group ($p \leq 0.05$). Administration of apigenin (10 and 20 mg/kg) significantly reduced the number of follicles ($p \leq 0.05$) (Figure 2).

Effect of different doses of apigenin on the number of graph follicles

The number of graph follicles in the PCOS group (group 2) did not change significantly compared to the control group. Administration of apigenin at different doses had no significant effect on the number of these follicles (Figure 3).

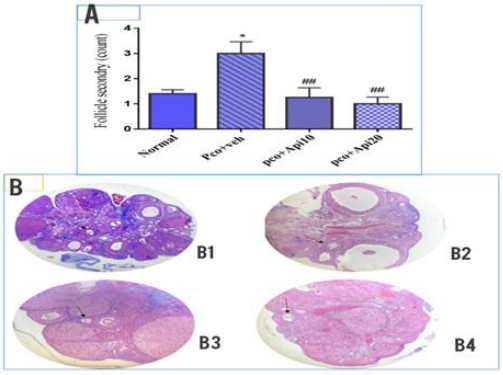

Figure 2- The effect of different doses of apigenin on secondary follicles in ovarian tissue of polycystic rats. A) The number of secondary follicles in the experimental groups. B) Tissue section of a polycystic ovary of a rat after Masson's trichrome staining (10× magnification). B1: control group, B2: PCOS group, B3: PCOS+ apigenin (10 mg/kg), B4: PCOS+ apigenin (20 mg/kg). * Significant difference compared to the control group. ## Significant difference compared with the PCOS+vehicle group

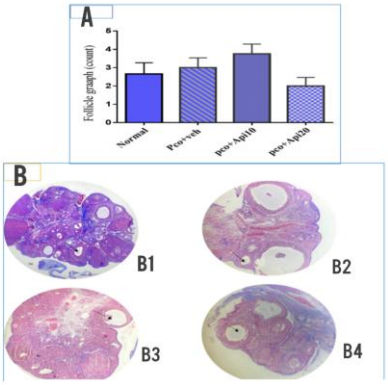

Figure 3- The effect of different doses of apigenin on graph follicles in ovarian tissue of polycystic rats. A) The number of primary follicles in the experimental groups. B) Tissue section of a polycystic ovary of a rat after Masson's trichrome staining (10× magnification). B1: control group, B2: PCOS group, B3: PCOS+ apigenin (10 mg/kg), B4: PCOS+ apigenin (20 mg/kg)

Effect of different doses of apigenin on the number of cystic follicles

The number of cystic follicles in the PCOS group (group2) was significantly increased compared to the control group ($p \leq 0.05$).

Administration of 10mg/kg apigenin significantly reduced the number of these follicles ($p \leq 0.05$). However, administration of apigenin at dose of 20 mg/kg had no significant effect on the d number of cystic follicles ($p \geq 0.05$) (Figure 4).

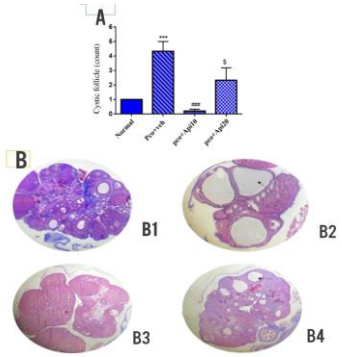

Figure 4- The effect of different doses of apigenin on cystic follicles in ovarian tissue of polycystic rats. A) The number of primary follicles in the experimental groups. *B)* Tissue section of a polycystic ovary of a rat after B1: control group, B2: PCOS group, B3: PCOS+ apigenin (10 Masson's trichrome staining (10× magnification). mg/kg), B4: PCOS+ apigenin (20 mg/kg). * Significant difference compared to the control group. ### Significant difference compared to the PCOS+Vehicle group.**

DISCUSSION

Recent examinations have pointed out that angiogenesis and fibrogenesis are involved in development of PCOS (2). Given the anti-angiogenic, antitumor, anti-proliferative, anti-cancer, and antioxidant effects of apigenin (8), we aimed to investigate the effects of apigenin on ovarian follicles in a rat model of PCOS. The results showed that with the induction of PCOS, the number of preantral and cystic follicles increased, but no significant change was seen in the number of graph follicles. This may be related to the fact that graph follicles are in the last stage of folliculogenesis; therefore, other doses of apegenin may affect graph follicles. Administration of apigenin was able to reduce the number of secondary follicles and cysts in the ovary.

It has been shown that ovarian function is affected by external factors such as nerves and hormones and intra-ovarian factors. Ovarian morphometric analysis showed that the induction of PCOS with estradiol valerate increased the total number of preantral follicles. In 2012, Abramovich et al. found that folliculogenesis is disrupted from the early stage to the preantral by a gonadotropin-independent transition. Preantral follicles are increased in women with PCOS but reduced in the ovaries of dehydroepiandrosterone-induced rats.

The preantral follicles might begin to grow in rats, and the growing follicles tend to become cystic rather than normal and turn into egg and corpus luteum. It has been shown that women with PCOS have more primordial, primary, and preantral follicles than normal women. In line with our findings, previous studies have shown that the total number of follicles increases in the ovaries of patients with PCOS (13, 14). In 2016, a study showed that gavage with letrozole (once daily for three weeks) increase the number of the cystic, secondary, and atresia follicles in PCOS, which is consistent with our findings (15). Previous studies also demonstrated that intramuscular injection of estradiol valerate results in a significant decrease in the number of primary and antral follicles and a significant increase in the number of cysts (16). In 2019, a study showed that intraperitoneal injection of estradiol valerate for three weeks increased cystic follicles but decreased the number of antral and corpus luteum follicles (12). In 2015, Bulut et al. reported that intraperitoneal injection of estradiol valerate (4 mg/kg) for 35 days significantly reduce the number of primary follicles, antral follicles, and corpus luteum. They also observed several large cystic follicles above the thin granulosa in the PCOS group. In this group, the high collagen density was reduced by c-Jun N-terminal Kinase, a stress-activated protein kinase induced by inflammatory cytokines, osmotic shock, UV radiation, and hypoxia (17).

In the present study, the effect of apigenin on tissue and molecular changes of polycystic ovaries were also investigated. We showed that in the primary follicles, administration of 10 and 20 mg/kg apigenin prevented the conversion of primordial follicles to primary follicles. It is recommended that 10 mg/kg apigenin reduces PCOS, but for ovulation stimulating, drugs such as clomiphene citrate is needed (18).

CONCLUSION

This study showed that apigenin, as an antioxidant and antiangiogenic agent, improves the complications of PCOS and exerts protective effects on ovarian follicles. Based on our findings, it is recommended to use apigenin for controlling or improving PCOS-related complications.

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DECLARATIONS

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Ethics approvals and consent to participate

The study was approved by the Ethics Committee and Deputy of Research and Development of Tehran University of Medical Sciences (ethical code: IR.TUMS.MEDICINE.REC.1398.915).

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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